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october 17, 1997
DATE

Attorney Docket No. P31353

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Lawlor, et al.	October 17, 1997
Serial No.:	08/785,455	Group Art Unit No.: 1814
Filed:	January 17, 1997	Examiner: Hobbs, L.
For:	NOVEL TRNA SYNTHETASE	

Assistant Commissioner of Patents
Washington, D.C. 20231

TRANSMITTAL OF CERTIFIED COPIES

Attached please find the certified copy of the foreign application from which priority is claimed for this case:

Country:	Great Britain
Application No.:	9601095.4
Filing Date:	19 January 1996; and

Country:	Great Britain
Application No.:	9615845.6
Filing Date:	27 July 1996

Respectfully submitted,

Edward R. Gimmi
Attorney for Applicant
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P31353

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9601095.4

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Request for grant of a Patent

Form 1/77 **Patents Act 1977**

❶ Title of invention

1 Please give the title of the invention: Novel Compounds

❷ Applicant's details

☐ **First or only applicant**

2a If you are applying as a corporate body please give:
Corporate Name: SMITHKLINE BEECHAM PLC

Country *(and State of incorporation, if appropriate)*: UNITED KINGDOM

2b If you are applying as an individual or one of a partnership please give in full:

Surname

Forenames

2c **In all cases,** please given the following details:

Address: NEW HORIZONS COURT
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Country: ENGLAND

ADP number *(if known)*: 05700971 4CC3

2d, 2e and 2f: If there are further applicants please provide details on a separate sheet of paper

☐ **Second applicant** ***(if any)***

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❶

please give details below

Agent's name **Connell A C**
Agent's address: CORPORATE INTELLECTUAL PROPERTY
SMITHKLINE BEECHAM PLC
SB HOUSE
GREAT WEST ROAD
BRENTFORD
MIDDLESEX

Postcode TW8 9BD
Agent's ADP number 200516700 2

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Name:

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❹ Reference number P31353

4. Agent's or applicant's reference number *(if applicable)*

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5. Are you claiming that this application be treated as having been filed on the date of filing of an earlier application?

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If you are declaring priority from a PCT Application please enter 'PCT' as the country and enter the country code (for example, GB) as part of the application number

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Inventorship

- any applicant is not an inventor
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❽ Checklist

8a Please fill in the number of sheets for each of the following types of document contained in this application

Continuation sheets for this Patents Form 1/77 ☐

Claim(s) **1** Description **15**

Abstract Drawing(s)

8b Which of the following documents also accompanies the application?

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Priority documents *(please state how many)* ☐

Translation(s) of Priority documents *(please state how many)* ☐

Patents Form 7/77 - Statement of Inventorship and Right to Grant ☐

Patents Form 9/77 - Preliminary Examination Report ☐

Patents Form 10/77 - Request for Substantive Examination ☐

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I/We request the grant of a patent on the basis of this application.

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Signed A C Connell Date: Thursday 18 January 1996
Dr A C Connell *(day month year)*

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NOVEL COMPOUNDS

This invention relates to a newly identified t-RNA synthetase isolated from a *S.aureus* organism, polynucleotides encoding for such a synthetase, the use of such synthetase and polynucleotides, as well as the production of such synthetase and polynucleotides and recombinant host cells transformed with the polynucleotides.

t-RNA synthetases have a primary role in protein synthesis according to the following scheme:

$$\text{Enzyme} + \text{ATP} + \text{AA} \Leftrightarrow \text{Enzyme.AA-AMP} + \text{PPi}$$

$$\text{Enzyme.AA-AMP} + \text{t-RNA} \Leftrightarrow \text{Enzyme} + \text{AMP} + \text{AA-t-RNA}$$

in which AA is an amino acid.

Inhibition of this process leads to a reduction in the levels of charged t-RNA and this triggers a cascade of responses known as the stringent response, the result of which is the induction of a state of dormancy in the organism. As such selective inhibitors of bacterial t-RNA synthetase have potential as antibacterial agents. One example of such is mupirocin which is a selective inhibitor of isoleucyl t-RNA synthetase. Other t-RNA synthetases are now being examined as possible anti-bacterial targets, this process being greatly assisted by the isolation of the synthetase.

Isoleucyl t-RNA synthetase, isolated from *Staphylococcus aureus,* has already been described (Chalker A F, Ward J M, Fosberry A P and Hodgson J E, 1994, Gene 141:103-108).

The present invention relates to a polypeptide isolated from *S.aureus* WCUH29, characterised in that it comprises the amino acid sequence given in SEQ ID NO 1, or a fragment, analogue or derivative thereof. This polypeptide is believed to be the enzyme methionyl t-RNA synthetase.

S.aureus WCUH29 has been deposited at the National Collection of Industrial and Marine Bacteria Ltd. (NCIMB), Aberdeen, Scotland under number NCIMB 40771 on 11 September 1995.

The invention also relates to the polypeptide having the amino acid sequence given in SEQ ID NO 1, or a derivative thereof.

Hereinafter the term polypeptide(s) will be used to refer to methionyl tRNA synthetase, fragments, analogues and derivatives thereof.

In accordance with another aspect of the present invention, there are provided polynucleotides (DNA or RNA) which encode such polypeptides.

In particular the invention provides a polynucleotide having the DNA sequence given in SEQ ID NO 2. The invention further provides a polynucleotide

encoding a polypetide from *S.aureus* WCUH29 and characterised in that it comprises the DNA sequence given in SEQ ID NO 2.

The polynucleotide having the DNA sequence given in SEQ ID NO 2 was obtained from a library of clones of chromosomal DNA of *S.aureus* WCUH29 in *E.coli*.

To obtain the polynucleotide encoding the polypeptide using the DNA sequence given in SEQ ID NO 2 typically a library of clones of chromosomal DNA of *S.aureus* WCUH29 in *E.coli* or some other suitable host is probed with a radiolabelled oligonucleotide, preferably a 17mer or longer, derived from the partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using high stringency washes. By sequencing the individual clones thus identified with sequencing primers designed from the original sequence it is then possible to extend the sequence in both directions to determine the full gene sequence. Conveniently such sequencing is performed using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook, J. in MOLECULAR CLONING, A Laboratory Manual [2nd edition 1989 Cold Spring Harbor Laboratory. see Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70].

The polynucleotide of the present invention may be in the form of RNA or in the form of DNA, which DNA includes cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encodes the polypeptide may be identical to the coding sequence shown in SEQ ID NO 2 or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptide.

The present invention includes variants of the hereinabove described polynucleotides which encode fragments, analogs and derivatives of the polypeptide characterised by the deduced amino acid sequence of SEQ ID NO 1. The variant of the polynucleotide may be a naturally occurring allelic variant of the polynucleotide or a non-naturally occurring variant of the polynucleotide.

Thus, the present invention includes polynucleotides encoding the same polypeptide characterised by the deduced amino acid sequence of SEQ ID NO 1 as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptide. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

The polynucleotide may have a coding sequence which is a naturally occurring allelic variant of the coding sequence characterised by the DNA sequence of SEQ ID NO 2. As known in the art, an allelic variant is an alternate form of a

polynucleotide sequence which may have a substitution, deletion or addition of one or more nucleotides, which does not substantially alter the function of the encoded polypeptide.

The polynucleotide which encodes for the mature polypeptide, i.e. the native methionyl tRNA synthetase, may include only the coding sequence for the mature polypeptide or the coding sequence for the mature polypeptide and additional coding sequence such as a leader or secretory sequence or a proprotein sequence.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention therefore includes polynucleotides, wherein the coding sequence for the mature polypeptide may be fused in the same reading frame to a polynucleotide sequence which aids in expression and secretion of a polypeptide from a host cell, for example, a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide from the cell. The polypeptide having a leader sequence is a preprotein and may have the leader sequence cleaved by the host cell to form the mature form of the polypeptide. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues. A mature protein having a prosequence is a proprotein and is an inactive form of the protein. Once the prosequence is cleaved an active mature protein remains.

Thus, for example, the polynucleotide of the present invention may encode for a mature protein, or for a protein having a prosequence or for a protein having both a prosequence and a presequence (leader sequence).

The polynucleotides of the present invention may also have the coding sequence fused in frame to a marker sequence at either the 5' or 3' terminus of the gene which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by the pQE series of vectors (supplied commercially by Quiagen Inc.) to provide for purification of the polypeptide fused to the marker in the case of a bacterial host.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 50% and preferably at least 70% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides . As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode

polypeptides which retain substantially the same biological function or activity as the polypeptide characterised by the deduced amino acid sequence of SEQ ID NO 1.

The deposit referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited material, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited material, and no such license is hereby granted.

The terms "fragment," "derivative" and "analog" when referring to the polypeptide characterised by the deduced amino acid sequence of SEQ ID NO 1, means a polypeptide which retains essentially the same biological function or activity as such polypeptide. Thus, an analog includes a proprotein which can be activated by cleavage of the proprotein portion to produce an active mature polypeptide.

The polypeptide of the present invention may be a recombinant polypeptide, a natural polypeptide or a synthetic polypeptide, preferably a recombinant polypeptide.

The fragment, derivative or analog of the polypeptide characterised by the deduced amino acid sequence of SEQ ID NO 1 may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the polypeptide, such as a leader or secretory sequence or a sequence which is employed for purification of the polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a

composition, and still be isolated in that such vector or composition is not part of its natural environment.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

In accordance with yet a further aspect of the present invention, there is therefore provided a process for producing the polypeptide of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host and recovering the expressed product. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a cosmid, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

Suitable expression vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTR or SV40 promoter, the *E. coli. lac* or *trp*, the phage lambda P_L promoter and other promoters known to control expression of genes in eukaryotic or prokaryotic cells or their viruses. The expression vector also contains a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying expression.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in *E. coli*.

The gene can be placed under the control of a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator (collectively referred to herein as "control" elements), so that the DNA sequence encoding the desired protein is transcribed into RNA in the host cell transformed by a vector containing this expression construction. The coding sequence may or may not contain a signal peptide or leader sequence. The polypeptides of the present invention can be expressed using, for example, the *E. coli* tac promoter or the protein A gene (spa) promoter and signal sequence. Leader sequences can be removed by the bacterial host in post-translational processing. See, e.g., U.S. Patent Nos. 4,431,739; 4,425,437; 4,338,397. Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are PKK232-8 and PCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In addition to control sequences, it may be desirable to add regulatory sequences which allow for regulation of the expression of the protein sequences relative to the growth of the host cell. Regulatory sequences are known to those of skill in the art, and examples include those which cause the expression of a gene to be turned on or off in response to a chemical or physical stimulus, including the presence of a regulatory compound. Other types of regulatory elements may also be present in the vector, for example, enhancer sequences.

An expression vector is constructed so that the particular coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and orientation of the coding sequence with respect to the control sequences being such that the coding sequence is transcribed under the "control" of the control sequences (i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence). Modification of the coding sequences may be desirable to achieve this end. For example, in some cases it may be necessary to modify the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame. The control sequences and other regulatory sequences may be ligated to the coding sequence prior to insertion into a vector, such as the cloning vectors described above. Alternatively, the coding sequence can be cloned directly into an expression vector which already contains the control sequences and an appropriate restriction site.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a

highly-expressed gene to direct transcription of a downstream structural sequence. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pET-3 vectors (Stratagene), pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks, pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pBlueBacIII (Invitrogen), pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Examples of recombinant DNA vectors for cloning and host cells which they can transform include the bacteriophage λ (*E. coli*), pBR322 (*E. coli*), pACYC177 (*E. coli*), pKT230 (gram-negative bacteria), pGV1106 (gram-negative bacteria), pLAFR1 (gram-negative bacteria), pME290 (non-*E. coli* gram-negative bacteria), pHV14 (*E. coli* and *Bacillus subtilis*), pBD9 (*Bacillus*), pIJ61 (*Streptomyces*), pUC6 (*Streptomyces*), YIp5 (*Saccharomyces*), a baculovirus insect cell system, , YCp19 (*Saccharomyces*). See, generally, "DNA Cloning": Vols. I & II, Glover *et al.* ed. IRL Press Oxford (1985) (1987) and; T. Maniatis *et al.* ("Molecular Cloning" Cold Spring Harbor Laboratory (1982).

In some cases, it may be desirable to add sequences which cause the secretion of the polypeptide from the host organism, with subsequent cleavage of the secretory signal.

Polypeptides can be expressed in host cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Depending on the expression system and host selected, the polypeptide of the present invention may be produced by growing host cells transformed by an expression vector described above under conditions whereby the polypeptide of interest is expressed. The polypeptide is then isolated from the host cells and purified. If the expression system secretes the polypeptide into growth media, the polypeptide can be purified directly from the media. If the polypeptide is not secreted, it is isolated from cell lysates or recovered from the cell membrane fraction. Where the polypeptide is localized to the cell surface, whole cells or isolated membranes can be used as an assayable source of the desired gene product. Polypeptide expressed in bacterial hosts such as *E. coli* may require isolation from inclusion bodies and refolding. Where the mature protein has a very hydroophobic region (normally at the C-terminus) which leads to an insoluble product of overexpression, it may be desirable to express a truncated protein in which the hydrophobic region has been deleted. The selection of the appropriate growth conditions and recovery methods are within the skill of the art.

The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-

glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

In a further aspect, the present invention further comprises a protein obtainable by expressing the polynucleotide having the DNA sequence given in SEQ ID NO 2.

A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

A "vector" is a replicon, such as a plasmid, phage, or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

A "double-stranded DNA molecule" refers to the polymeric form of deoxyribonucleotides (bases adenine, guanine, thymine, or cytosine) in a double-stranded helix, both relaxed and supercoiled. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having the sequence homologous to the mRNA).

A DNA "coding sequence of" or a "nucleotide sequence encoding" a particular protein, is a DNA sequence which is transcribed and translated into a polypeptide when placed under the control of appropriate regulatory sequences.

A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bound at the 3' terminus by a translation start codon (e.g., ATG) of a coding sequence and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

DNA "control sequences" refers collectively to promoter sequences, ribosome binding sites, polyadenylation signals, transcription termination sequences, upstream

regulatory domains, enhancers, and the like, which collectively provide for the expression (i.e., the transcription and translation) of a coding sequence in a host cell.

A control sequence "directs the expression" of a coding sequence in a cell when RNA polymerase will bind the promoter sequence and transcribe the coding sequence into mRNA, which is then translated into the polypeptide encoded by the coding sequence.

A "host cell" is a cell which has been transformed or transfected, or is capable of transformation or transfection by an exogenous DNA sequence.

A cell has been "transformed" by exogenous DNA when such exogenous DNA has been introduced inside the cell membrane. Exogenous DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes and yeasts, for example, the exogenous DNA may be maintained on an episomal element, such as a plasmid. With respect to eukaryotic cells, a stably transformed or transfected cell is one in which the exogenous DNA has become integrated into the chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cell containing the exogenous DNA.

A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

A "heterologous" region of a DNA construct is an identifiable segment of DNA within or attached to another DNA molecule that is not found in association with the other molecule in nature.

In accordance with another aspect of the present invention, there are provided inhibitors to such polypeptides which are useful as antibacterial agents.

This invention also provides a pharmaceutical or veterinary composition which comprises an inhibitor of the present invention (hereinafter referred to as the 'drug') together with a pharmaceutically or veterinarily acceptable carrier or excipient. The compositions may be formulated for administration by any route, and would depend on the disease being treated. The compositions may be in the form of, for instance, tablets, capsules, powders, granules, suppositories, lozenges and liquid or gel preparations, including oral, topical and sterile parenteral suspensions.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinyl-pyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch, or acceptable wetting agents such as

sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, glucose syrup, gelatin, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid, and if desired conventional flavouring or colouring agents.

For topical application to the skin the drug may be made up into a cream, lotion or ointment. Cream or ointment formulations that may be used for the drug are conventional formulations well known in the art, for example, as described in standard text books of pharmaceutics and cosmetics, such as Harry's Cosmeticology, 7th edn, ed Wilkinson and Moore, 1982, George Godwin, Harlow, England and the British Pharmacopoeia.

Suppositories will contain conventional suppository bases, e.g. cocoa-butters or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the drug and a sterile vehicle. The drug, depending on the vehicle and concentration used, can be suspended in the vehicle. Advantageously, adjuvants such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability the composition can be frozen after filling into the vial and water removed under vacuum. The dry lypophilized powder is then sealed in the vial. The drug can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the drug.

For topical application to the ear, the drug may be made up into a suspension in a suitable liquid carrier, such as water, glycerol, diluted ethanol, propylene glycol, polyethylene glycol or fixed oils. For topical application to the eye, the drug is formulated as a suspension in a suitable, sterile aqueous or non-aqueous vehicle. Additives, for instance buffers such as sodium metabisulphite or disodium edetate; preservatives including bactericidal and fungicidal agents, such as phenylmercuric acetate or nitrate, benzalkonium chloride or chlorhexidine, and thickening agents such as hypromellose may also be included.

The dosage employed for compositions administered topically will, of course, depend on the size of the area being treated. For the ears and eyes each dose will typically be in the range from 10 to 100 mg of the drug.

Veterinary compositions for intramammary treatment of mammary disorders in animals, especially bovine mastitis, will generally contain a suspension of the drug in an oily vehicle.

The compositions may contain from 0.1% to 99% by weight, preferably from 10-60% by weight, of the drug, depending on the method of administration. Where the compositions are in unit dose form, each dosage unit will preferably contain from 50-500 mg, of the drug. The dosage as employed for adult human treatment (average weight about 70 kg) will preferably range from 100 mg to 3 g per day, for instance 250 mg to 2 g of the drug per day, depending on the route and frequency of administration. Alternatively, the drug may be administered as part of the total dietary intake of a non-human animal. In this case the amount of drug employed may be less than 1% by weight of the diet and in preferably no more than 0.5% by weight. The diet for animals may consist of normal foodstuffs to which the drug may be added or the drug may be included in a premix for admixture with the foodstuff. A suitable method of administration of the drug to animals is to add it to the non-human animal's drinking water. In this case a concentration of the drug in the drinking water of about 5-500 mg/ml, for example 5-200 mg/ml, is suitable.

In a further aspect, this invention provides a method of screening drugs to identify those which interfere with the interaction of the methionyl tRNA synthetase. The enzyme mediated incorporation of radiolabelled amino acid into tRNA may be measured by the aminoacylation method which measures amino acid-tRNA as trichloroacetic acid-precipitable radioactivity from radiolabelled amino acid in the presence of tRNA and ATP (Hughes J, Mellows G and Soughton S, 1980, FEBS Letters, 122:322-324). Thus inhibitors of methionyl tRNA synthetase can be detected by a reduction in the trichloroacetic acid precipitable radioactivity relative to the control. Alternatively the tRNA synthetase catalysed partial PPi/ATP exchange reaction which measures the formation of radiolabelled ATP from PPi can be used to detect methionyl tRNA synthetase inhibitors (Calender R & Berg P, 1966, Biochemistry, 5, 1681-1690).

In order to facilitate understanding of the following example certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case 'p' preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction

enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 µg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 µl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 µg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37 C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 µg of approximately equimolar amounts of the DNA fragments to be ligated.

Example 1

Isolation of DNA coding for a tRNA Synthetase from *S.aureus* WCUH29

The polynucleotide having the DNA sequence given in SEQ ID NO 2 was obtained from a library of clones of chromosomal DNA of *S.aureus* WCUH29 in *E.coli*. Libraries may be prepared by routine methods, for example:

Methods 1 and 2

Total cellular DNA is isolated from *S.aureus* strain WCUH29 (NCIMB 40771) according to standard procedures and size-fractionated by either of two methods.

Method 1.

Total cellular DNA is mechanically sheared by passage through a needle in order to size-fractionate according to standard procedures. DNA fragments of up to 11kbp in size are rendered blunt by treatment with exonuclease and DNA polymerase, and EcoRI linkers added. Fragments are ligated into the vector Lambda ZapII that has been cut with EcoRI, the library packaged by standard procedures and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Method 2.

Total cellular DNA is partially hydrolsed with a combination of four restriction enzymes (RsaI, PalI, AluI and Bsh1235I) and size-fractionated according to standard procedures. EcoRI linkers are ligated to the DNA and the fragments then ligated into the vector Lambda ZapII that have been cut with EcoRI, the library packaged by standard procedures, and *E.coli* infected with the packaged library. The library is amplified by standard procedures.

Sequence Listing for *S. aureus* Methionyl tRNA Synthetase

SEQ ID NO 1

```
  1  YYTESQLXDP QYENGKIIGG XSPDSGHEVE LVKEESYFFN ISKYTDRLLE
 51  FYDQNPDFIQ PPSRKNEMIN NXIKPGLADL AVSRTSFNWG VHVPSNPKHV
101  VYVWIDAL
```

SEQ ID NO 2

```
  1  TACTATACAG AGTCACAATT AGNAGACCCA CAATACGAAA ACGGTAAAAT
 51  TATTGGTGGC ANAAGTCCAG ATTCTGGACA CGAAGTTGAA CTAGTTAAAG
101  AAGAAAGTTA TTTCTTTAAT ATTAGTAAAT ATACAGACCG TTTATTAGAG
151  TTCTATGACC AAAATCCAGA TTTTATACAA CCACCATCAA GAAAAAATGA
201  AATGATTAAC AACTNCATTA AACCAGGACT TGCTGATTTA GCTGTTTCTC
251  GNACATCATT TAACTGGGGT GTCCATGTTC CGTCTAATCC AAAACATGTT
301  GTTTATGTTT GGATTGATGC GTTA
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Claims

1. A polypeptide isolated from *S.aureus* WCUH29, characterised in that it cnsists of the amino acid sequence given in SEQ ID NO 1, or a fragment, analogue or derivative thereof.

2. A polypeptide having the amino acid sequence given in SEQ ID NO 1, or a derivative thereof.

3. A polynucleotides (DNA or RNA) which encodes a polypeptide according to claim 1 or 2.

4. A polynucleotide having the DNA sequence given in SEQ ID NO 2.

5. A polypetide from *S.aureus* WCUH29 characterised in that it comprises the DNA sequence given in SEQ ID NO 2.

6. A protein obtainable by expressing the polynucleotide having the DNA sequence given in SEQ ID NO 2.

7. An inhibitor of the polypeptide according to claim 1.

8. A pharmaceutical composition comprising an inhibitor as claimed in claim 7 and a pharmaceutically acceptable carrier or excipient.

9. The use of an inhibitor according to claim 7 in therapy.

10. A method of screening drugs to identify those which interfere with the interaction of the methionyl tRNA synthetase which method comprises measurement of enzyme activity by the full aminoacylation reaction or the partial PPi/ATP exchange reaction.